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SEM observations on the seed surface of *Hyacinthaceae*

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with 5 figures and 5 tables

Key words: *Hyacinthaceae* – Seeds, testa, micromorphology, scanning electron microscopy (SEM).

Summary

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Data on seed morphology of 132 species from 40 genera of all subfamilies (*Ornithogaloideae*, *Hyacinthoideae, Urgineoideae*, *Oziroëoideae*) of *Hyacinthaceae* are presented. So far, this is the most extensive study on the seed surface of *Hyacinthaceae* using scanning electron microscopy (SEM), and we also give insights into the systematic implications of seed surface micromorphology.

1. Introduction

The family *Hyacinthaceae* (sensu APG 2003) contains about 700–1000 species of bulbous plants distributed in Africa, Europe and Asia. With *Oziroë* (Rafinesque 1837: 53) *Hyacinthaceae* are also occuring in South America (Speta 1998a, b, APG 2003). In APG 2009 and APG 2016, *Hyacinthaceae* are treated as *Asparagaceae* subfamily *Scilloideae* and the former accepted subfamilies *Hyacinthoideae*, *Ornithogaloideae*, *Oziroëoideae* and *Urgineoideae* (Pfosser & Speta 1999, APG 2003, Manning & al. 2004, Martínez-Azorín & al. 2011) are reduced to tribes. However, we favor *Hyacinthaceae* for morphological reasons.

Comprehensive studies on seed micromorphology covering all subfamilies in *Hyacinthaceae* are not available. Jessop 1975 defined three tribes of South African *Liliaceae* – *Scilleae*, *Massonieae* and *Bowieae* – and shows the importance of seed morphology for generic circumscription based on seed surface traits. Moret & al. 1990, Coşkunçelebi & al. 2000 and Bednorz & Czarna 2008 presented detailed studies on seed surface morphology in species of *Ornithogalum* s.str. The works of Wetschnig & al. 2002 and Pfosser & al. 2003 give good insights into variability of general seed morphology in *Massonieae* but detailed data on seed micromorphology are lacking. Luidold 2010 and Koller 2010, in their unpublished master theses, provide the first accurate comprehensive information on seed micromorphology in *Ornithogaloideae*, *Urgineoideae* and *Oziroëoideae*. Recently, Brudermann & al. 2018 compared the seed micromorphology of *Rhodocodon* species.

Most of micromorphological research on plant epidermals in the last three decades is based on the works of Barthlott & Ehler 1977 and Barthlott 1981, 1984. Barthlott & Ehler 1977 examined epidermal surfaces of about 2100 Angiosperms and about 45 Gymnosperms with scanning electron microscopy and classified the microscopic diversity of their surface sculptures and structures in three basic categories:

- the arrangement of cells and distribution of idioblastic elements
- the form of single cells
- the sculpturing of the cell wall surface

In this paper, data on seed morphology of 132 species from 40 genera of all subfamilies (19 *Ornithogaloideae*, 4 *Hyacinthoideae,* 16 *Urgineoideae*, 1 *Oziroëoideae*) of *Hyacinthaceae* are presented. We focus on South African taxa that include the highest diversity of genera and species in the family and complement it with material from the whole family

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distribution range from Europe, Asia and South America. To our knowledge, this study is the most extensive work on seed surface characteristics of *Hyacinthaceae* published so far. We here present the results of the scanning electron microscopic examination, with an evaluation of 11 characters, and give insights into the systematic implications of seed surface micromorphology.

2. Material and methods

Samples (Table 1) were taken from the living plant collection cultivated at the Botanical Garden, Institute of Biology, University of Graz, the private collections of M. Martínez-Azorín and W. Knirsch, as well as some samples from seed trade. Seeds were stored in the *Hyacinthaceae* seed collection of W. Wetschnig.

The focus of the present study was on the *Ornithogaloideae*. Selected genera of *Hyacinthoideae* and *Urgineoideae* were only examined for comparison, therefore the data and information from these selected genera are not representative for their subfamilies.

Two to five representative seeds from one plant per species were used. Air dried seeds were mounted on aluminum stubs using double-sided adhesive tapes and sputter coated with gold in an AGAR sputter coater. Micromorphology was studied using a Phillips XL30 ESEM operated at 20 kV in the high vacuum mode. Images were analyzed using the program Axio Vision SE 64.

For evaluation of cell width and length, frames were applied tangentially on the cells by which the four outermost points of each side were connected in a rectangle. All measurements are average values.

The morphological terminology from Barthlott & Ehler 1977, Barthlott 1981, 1984 and Werker 1997 was used and modified when necessary. Following Barthlott & Ehler 1977: 30, the vault ratio of the periclinal outer cell walls was divided by the quotient of width and height into the following categories: convex, hemispheric, domed, papillate, hair-papillate, and trichome.

Table 1. Voucher data for specimens of *Hyacinthaceae* used in this study. – Subfamilies: Orn *Ornithogaloideae*, Hya *Hyacinthoideae*, Urg *Urgineoideae*, Ozi *Oziroëoideae*. – Countries of origin: AUT Austria, CHL Chile, ESP Spain, FRA France, ITA Italy, MAR Morocco, MDG Madagascar, NAM Namibia, TUR Turkey, ZAF South Africa (n.d. = no data).

Seed voucher: sample number in the *Hyacinthaceae* seed collection kept in the Institute of Biology, University of Graz. – Plant voucher: specimen numbers in the collections of W. Wetschnig (without initials) and M. Martínez-Azorín (MA). – Herbarium Alicante ID: specimen numbers in the herbarium of the University of Alicante (Index Herbariorum acronym: ABH).

Species	Subfamily	Seed voucher	Plant voucher	Herbarium Alicante ID	Country
Albuca angolensis	Orn	02618			ZAF
Albuca canadensis	Orn	03775			ZAF
Albuca clanwilliamae-gloria	Orn	02625			ZAF
Albuca cf. *juncifolia*	Orn	02574	5202-01		ZAF
Albuca maxima	Orn	03786			ZAF
Albuca nelsonii	Orn	03780			ZAF
Albuca paradoxa	Orn	05122	MA289	58957	ZAF
Albuca shawii	Orn	05123	MA21	58794	ZAF
Albuca tenuifolia	Orn	05124	MA180	58848	ZAF
Albuca tortuosa	Orn	05125	MA80		ZAF
Albuca spec.	Orn	02436			MDG
Avonsera convallarioides	Orn	02627			MDG
Battandiera amoena	Orn	05126			MAR
Battandiera angolensis	Orn	05127			NAM
Battandiera stapffii	Orn	05128	MA578	58708	NAM
Cathissa concinna	Orn	05129		47141	ESP
Cathissa reverchonii	Orn	05130		47138	ESP

Species	Subfamily	Seed voucher	Plant voucher	Herbarium Alicante ID	Country
Cathissa unifolia	Orn	05131		50131	ESP
Coilonox concordianum	Orn	03730			n.d.
Coilonox diluculum	Orn	05132	MA427	58902	ZAF
Coilonox namaquanum	Orn	05133	MA423	58893	ZAF
Coilonox paucifolium	Orn	05134	MA816	59762	ZAF
Coilonox pearsonii	Orn	05135	MA671	59557	ZAF
Coilonox secundum	Orn	03768			ZAF
Coilonox vittatum	Orn	05136	MA397	58881	ZAF
Coilonox spec.	Orn	02583	52208-01		ZAF
Dipcadi bakerianum	Orn	05137	MA576	58704	NAM
Dipcadi ciliare	Orn	05138	MA25	58750	ZAF
Dipcadi crispum	Orn	05139	MA598		ZAF
Dipcadi glaucum	Orn	05140	MA577	58707	NAM
Dipcadi marlothii	Orn	02629			ZAF
Dipcadi serotinum	Orn	05141			ESP
Dipcadi viride	Orn	05142			ZAF
Eliokarmos conicus	Orn	02624			ZAF
Eliokarmos constrictus	Orn	05145	MA907	73944	ZAF
Eliokarmos craibii	Orn	05143	MA1669	74372	ZAF
Eliokarmos decus-montium	Orn	05146	MA670	59559	ZAF
Eliokarmos dubius	Orn	03655			ZAF
Eliokarmos cf. *multifolius*	Orn	02580	52234-01		ZAF
Eliokarmos pilosus	Orn	05147	MA446	58935	ZAF
Eliokarmos cf. *pilosus*	Orn	02559	52158-01		ZAF
Eliokarmos synanthifolius	Orn	05149	MA7	58757	ZAF
Eliokarmos thunbergianus	Orn	05148	MA257	58973	ZAF
Eliokarmos thunbergianus	Orn	03763			ZAF
Eliokarmos thyrsoides	Orn	02626			ZAF
Ethesia prasina	Orn	05150	MA567	58702	NAM
Ethesia tanquana	Orn	05151	MA815	59763	ZAF
Ethesia xanthochlora	Orn	03771			
Galtonia candicans	Orn	05152	MA917	59466	ZAF
Galtonia princeps	Orn	02684	01538		ZAF
Galtonia regalis	Orn	02634			ZAF
Galtonia saundersiae	Orn	03656			ZAF
Galtonia viridiflora	Orn	05154	MA922		ZAF
Honorius nutans	Orn	02561			n.d.
Igidia erecta ined.	Orn	04970	04452		MDG

Species	Subfamily	Seed voucher	Plant voucher	Herbarium Alicante ID	Country
Igidia volubilis	Orn	05039	04351-02		MDG
Loncomelos kayiranii ined.	Orn	05155			TUR
Loncomelos narbonense	Orn	05156		47144	ESP
Loncomelos pyrenaicum	Orn	02562			n.d.
Melomphis arabica	Orn	05157		50142	ESP
Neopatersonia falcata	Orn	05158	MA701	59520	ZAF
Neopatersonia rotata	Orn	05159			n.d.
Neopatersonia uitenhagensis	Orn	04158	04018		ZAF
Nicipe capillaris	Orn	05161	MA167	58974	ZAF
Nicipe comptum	Orn	05162	MA474	58748	ZAF
Nicipe flexuosa	Orn	05163	MA871	59460	ZAF
Nicipe graminifolia	Orn	03731			n.d.
Nicipe hesperantha	Orn	05164	MA800	59744	ZAF
Nicipe pilosa	Orn	05165	MA439	58946	ZAF
Nicipe sardienii	Orn	03767			ZAF
Nicipe tubiflora ined.	Orn	05166	MA24	58751	ZAF
Nicipe zebrinella	Orn	05167	MA850	59790	ZAF
Ornithogalum baeticum	Orn	05168		47135	ESP
Ornithogalum balansae	Orn	02563			n.d.
Ornithogalum bourgaeanum	Orn	05169		47142	ESP
Ornithogalum comosum	Orn	03686			n.d.
Ornithogalum divergens	Orn	05170		52828	FRA
Ornithogalum kochii	Orn	02565			AUT
Pseudogaltonia clavata	Orn	02384			n.d.
Pseudogaltonia liliiflora	Orn	05171			ZAF
Stellarioides exigua	Orn	05175	MA106		ZAF
Stellarioides flavovirens	Orn	05172	MA196	58768	ZAF
Stellarioides gigantea ined.	Orn	05173	MA114		ZAF
Stellarioides inconspicua	Orn	05174	MA142	58871	ZAF
Stellarioides longibracteata	Orn	03653			ZAF
Stellarioides sessiliflora	Orn	05176		58399	MAR
Stellarioides tenuifolia	Orn	03654			ZAF
Trimelopter unifolium	Orn	03770			ZAF
Trimelopter spec.	Orn	05178	MA182	59468	ZAF
Autonoë spec.	Hya	02374	02224		NAM
Barnardia chinensis	Hya	02373			n.d.
Barnardia numidica	Hya	02371			n.d.
Barnardia scilloides	Hya	02385			n.d.

Species	Subfamily	Seed voucher	Plant voucher	Herbarium Alicante ID	Country
Chouardia litardierei	Hya	02387			n.d.
Chouardia pratensis	Hya	02377			n.d.
Schnarfia messeniaca	Hya	02388			n.d.
Boosia macrocentra	Urg	02642			ZAF
Bowiea volubilis	Urg	02260	02259		ZAF
Charybdis maritima	Urg	02590			n.d.
Drimia elata	Urg	02641			ZAF
Drimia elata	Urg	02692			ZAF
Fusifilum capitatum	Urg	02691			ZAF
Litanthus pusillus	Urg	03638			ZAF
Mucinaea nana	Urg	02609			ZAF
Rhadamanthopsis namibensis	Urg	03640			NAM
Rhadamanthus convallarioides	Urg	02602			ZAF
Rhadamanthus platyphyllus	Urg	03675	02458-03		ZAF
Rhodocodon campanulatus	Urg	04582	02446-02		MDG
Rhodocodon cryptopodus	Urg	04686	02416-06		MDG
Rhodocodon cyathiformis	Urg	04679	04479-05		MDG
Rhodocodon floribundus	Urg	04683	04478-05		MDG
Rhodocodon giganteus	Urg	03843	02514-01		MDG
Rhodocodon graciliscapus	Urg	05025	04477-07		MDG
Rhodocodon intermedius	Urg	04682	04475-05		MDG
Rhodocodon jackyi	Urg	04684	04669-06		MDG
Rhodocodon mascarenensis	Urg	03708			MDG
Rhodocodon monophyllus	Urg	04778	04677-01		MDG
Rhodocodon perrieri	Urg	04774	04567-01		MDG
Rhodocodon rotundus	Urg	04586	02407-01		MDG
Rhodocodon urgineoides	Urg	02432			MDG
Schizobasis intricata	Urg	03699			n.d.
Sekanama sanguinea	Urg	02643			ZAF
Tenicroa filifolia	Urg	02606			ZAF
Thuranthos basuticum	Urg	03755			ZAF
Urginavia altissima	Urg	02640			ZAF
Urginavia capensis	Urg	03739			ZAF
Urginea fugax	Urg	03742			ITA
Urginea modesta	Urg	03756			ZAF
Urginea tenella	Urg	03649			ZAF
Oziroë arida	Ozi	02438			CHL
Oziroë biflora	Ozi	02439			CHL

3. Results

The results were grouped on subfamily or tribe level to provide a better overview. Variations in seed characters in *Hyacinthaceae* are shown in Tables 2 and 3. Tables 4 and 5 refer only to the subfamily *Ornithogaloideae*. All tables show absolute values. Some characters can be found combined on a single seed. Some values could not be obtained at all, due to a thick layer of wax or, in very rare cases, due to lack of seed material.

Table 2. Primary seed surface sculpture of *Hyacinthaceae* (Orn *Ornithogaloideae*, Hya *Hyacinthoideae*, Urg *Urgineoideae*, Ozi *Oziroëoideae*). Numbers indicate the number of species showing each character.

				Subfamily	**Orn**	**Hya**	**Urg**	**Ozi**
primary sculpture	outlines of epidermal cells			elongate-polygonal	70	2	19	1
				elongate-tetragonal/polygonal	3	0	2	0
				isodiametric/elongate-tetragonal/polygonal	2	1	0	0
				isodiametric/elongate-polygonal	15	2	7	1
				isodiametric-polygonal	0	2	5	0
	distribution			consistent	88	7	33	2
				inconsistent	6	0	1	0
	anticlinal cell walls			straight	22	7	14	2
				U-undulated	4	0	0	0
				Ω-undulated	1	0	0	0
				curved	6	1	9	2
				irregularly lobate	62	0	16	1
	anticlinal cell boundaries			raised	15	5	9	0
				sunken	76	2	20	2
				flat	2	0	4	0
	cell corners			not divergent	89	6	33	2
	curvatures of periclinal outer cells	plane			5	1	13	0
		convex		convex	31	0	9	0
				convex or papillate	1	0	0	0
				hemispheric	8	0	1	2
				hemispheric or domed	3	0	0	0
				domed	4	0	0	0
				domed or papillate	5	0	0	0
				papillate	5	0	0	0
				papillate or hair-papillate	2	0	0	0
				hair-papillate	1	0	0	0
				hair-papillate or trichome	1	0	0	0
				trichome	1	0	0	0
		concave		concave	10	0	13	0
				concave-cohesion deformed	6	1	0	0
				cohesion deformed	9	5	6	0
				several collapsed papillae	1	0	0	0

Table 3. Secondary and tertiary seed surface scuplture of *Hyacinthaceae* (Orn *Ornithogaloideae*, Hya *Hyacinthoideae*, Urg *Urgineoideae*, Ozi *Oziroëoideae*). Numbers indicate the number of species showing each character.

		Subfamily	Orn	Hya	Urg	Ozi
secondary sculpture	surface of the outer cell wall	smooth	36	0	13	0
		foveolate	4	0	4	0
		verrucous	46	3	11	0
		reticulate	4	0	4	0
		striate	3	0	0	0
		cohesion deformed	19	3	3	0
		folded	19	1	3	0
tertiary sculpture	wax type	plane-smooth	4	0	8	0
		plane-verrucous	9	1	3	0
		plane-discontinuous	1	0	0	0
		plane-granulous	5	0	0	0
		recumbent rods	1	0	1	2
		platelets	0	0	2	0
		blisters	1	0	0	0
		helical rods	0	0	1	2
stomata			1	0	0	2

3.1. Primary sculpture

Outlines of epidermal cells are tetra- or polygonal, combined with isodiametric to elongate. Barthlott & Ehler 1977 described four types which can be defined as:

a. isodiametric-tetragonal (Fig. 1a)
b. elongate-tetragonal (Fig. 1b)
c. isodiametric-polygonal (Fig. 1c)
d. elongate-polygonal (Fig. 1d)

In seeds of *Hyacinthaceae*, the elongate-polygonal type dominates (Table 2). This cell type occurred in 92 (70 %) of the 132 examined species. Isodiametric-polygonal cells were found in only 5 *Urgineoideae* and 2 *Hyacinthoideae* species, and they are apparently absent in *Ornithogaloideae*. Interestingly, we identified three species with all cell types mentioned above (a–d) homogeneously distributed over the whole seed surface: *Ornithogalum baeticum*, *O. comosum* (*Ornithogaloideae*), and *Schnarfia messeniaca* (*Hyacinthoideae*).

Koller 2010 discussed the distribution of cell types on a single seed and divided the seed in three regions: hilum, central and peripheral. The differentiation of cells in these regions follows the same principles in all investigated seeds: the hilum region is characterized by dense isodiametric cells (Fig. 1e1) which increase in size towards to the central region (Fig. 1e2). Cells at the hilum region show elongate or elongate to isodiametric cells (Fig. 1e3). If the cell distribution follows this pattern, then the distribution is considered as consistent and any subsequent remarks, especially the characteristics of the primary structure, pertain to cells of the central region.

In contrast to these forms of consistent distribution, we also found a distinctly more varying distribution of cell types over the whole surface in seven species, six of them in *Ornithogaloideae* and one in *Urgineoideae*. Further research might show a possible function of these conspicuously divergent cell patterns.

Anticlinal walls are straight (Fig. 2a–c), curved, or more or less undulate. Curved anticlinal walls never appear alone and are always found in combination with straight ones (Fig. 2d–f). This applies to all investigated species in *Ornithogalum* L. (Fig. 2f). Only 5 species showed U-undulate (Fig. 3a, b) or Ω-undulate (Fig. 3c) anticlinal walls. We detected two species with plicate margins that Barthlott & Ehler 1977 defined as V-undulate. Unlike Barthlott & Ehler's conclusion, the V-undulate margins were too infrequent to confirm them as a separate feature. Most of the species show these ir-

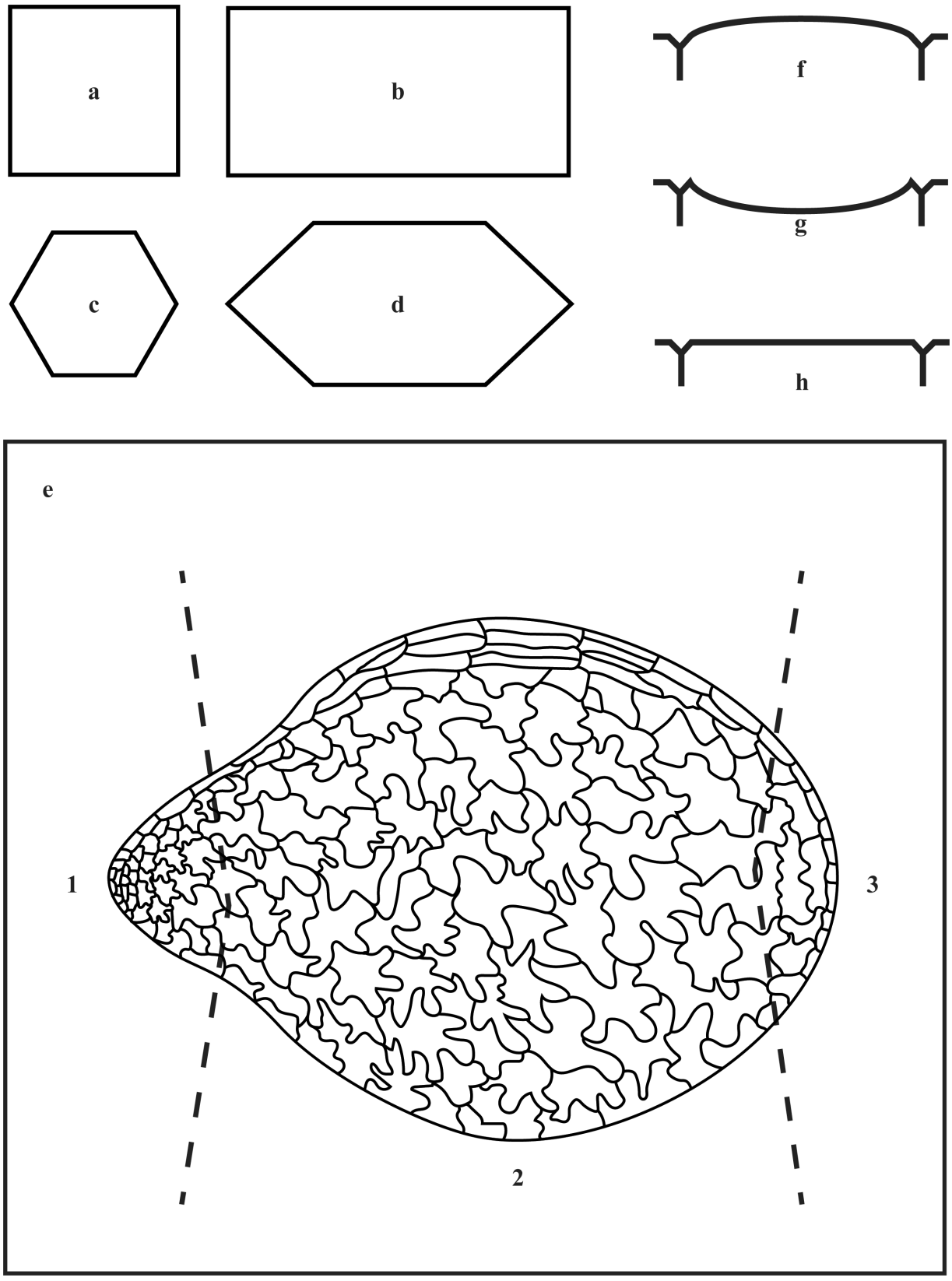

Fig. 1. (a–h) Schematic diagrams of seed structures of *Hyacinthaceae*: (a–d) Cells in top view. – (e) Seed in longitudinal view (1 hilum region, 2 central region, 3 peripheral region). – (f–h) Curvatures of outer periclinal cell walls. – (a) isodiametric-tetragonal, (b) elongate-tetragonal, (c) isodiametric-polygonal, (d) elongate-polygonal, (f) convex, (g) concave, (h) flat.

Table 4. Primary seed surface sculpture of subfamily *Ornithogaloideae* (Orn *Ornithogaleae*, Alb *Albuceae*, Dip *Dipcadieae*). Numbers indicate the number of species showing each character.

				Tribus	Orn	Alb	Dip
primary sculpture	outlines of epidermal cells			elongate-polygonal	36	32	2
				elongate-tetragonal/polygonal	2	1	0
				isodiametric/elongate-tetragonal/polygonal	2	0	0
				isodiametric/elongate-polygonal	8	1	6
	distribution			consistent	47	33	8
				inconsistent	4	1	1
	anticlinal cell walls			straight	13	1	8
				U-undulated	4	0	0
				Ω-undulated	1	0	0
				curved	6	0	0
				irregularly lobate	29	33	0
	anticlinal cell boundaries			raised	13	1	2
				sunken	36	34	6
				flat	0	1	1
	cell corners			not divergent	48	33	8
	curvatures of periclinal outer cells	plane			0	4	1
		convex		convex	18	12	1
				convex or papillate	0	1	0
				hemispheric	2	6	0
				hemispheric or domed	1	2	0
				domed	2	2	0
				domed or papillate	2	3	0
				papillate	3	3	0
				papillate or hair-papillate	2	0	0
				hair-papillate	1	0	0
				hair-papillate or trichome	1	0	0
				trichome	1	0	0
		concave		concave	5	2	3
				concave-cohesion deformed	5	0	1
				cohesion deformed	5	0	4
				several collapsed papillae	1	0	0

Table 5. Secondary and tertiary seed surface sculpture of subfamily *Ornithogaloideae* (Orn *Ornithogaleae*, Alb *Albuceae*, Dip *Dipcadieae*). Numbers indicate the number of species showing each character.

		Tribus	**Orn**	**Alb**	**Dip**
secondary sculpture	surface of the outer cell wall	smooth	28	7	1
		foveolate	0	2	2
		verrucous	18	22	6
		reticulate	0	2	2
		striate	3	0	0
		cohesion deformed	15	3	1
		folded	17	2	0
tertiary sculpture	wax type	plane-smooth	1	2	1
		plane-verrucous	5	0	4
		plane-discontinuous	1	0	0
		plane-granulous	5	0	0
		recumbent rods	1	0	0
		blisters	1	0	0
stomata			1	0	0

regularly undulate anticlinal walls, so we designate this type as irregularly lobate (Fig. 3e) which was, after straight, the second most common character (Table 2) but never found in *Hyacinthoideae*.

Anticlinal cell boundaries are most commonly channelled or sunken (Fig. 3c), more rarely raised (Fig. 3d). It should be noted that SEM is not always suitable for this character, especially for strongly convex cells (Fig. 3e). Their curvature at the cell boundaries only rarely allow a clear statement about the nature of the grooves.

Curvatures of periclinal outer cell walls may be convex (Fig. 1f), concave (Fig. 1g), or plane (Fig. 1h). Convex cells are very diverse, being very common in *Ornithogaloideae*, rare in *Oziroëoideae* and *Urgineoideae*, and absent in *Hyacinthoideae*. According to Barthlott & Ehler 1977: 30, we determined convex (Fig. 2d, 3f), hemispheric (Fig. 4a), domed (Fig. 3e), papillate (Fig. 3b), hair-papillate (Fig. 4b), and trichome (Fig. 4c). In contrast, concave outer cell walls are very common in *Ornithogaloideae* and *Urgineoideae*. Most of the cells show collapsed (Barthlott & Ehler 1977: 33) cells (Fig. 2c, e, f) or just concave shapes (Fig. 2b, 4c), except in *Cathissa reverchonii* (Lange) Speta. This species shows several collapsed papillae (Fig. 4e), which are unique in the *Hyacinthaceae* selected for the present study.

It should be noted here that these types of periclinal cell walls can also occur mixed on the entire seed surface.

3.2. Secondary sculpture

The surface of the outer seed cell wall is manifold in *Hyacinthaceae*. Half of all investigated species are characterized by verrucous structures (Fig. 2c, 3d), followed by smooth surfaces (Fig. 3c, 3e). Reticulate (Fig. 2b) structures were less frequent in *Ornithogaloideae* and *Urgineoideae*. Striate surfaces (Fig. 4f) occur only in *Ornithogaloideae*; possibly this structure can be attributed to some of the cuticular folds described in detail by Barthlott & Ehler 1977.

Cuticular folds (Fig. 3b, 4b, 4c) were observed in all subfamilies except in *Oziroëoideae*. In contrast to these folding patterns, we also observed irregular surfaces which were presumably caused by the natural dehydration process and assigned to the character 'compression-deformed' (Fig. 2e, 2f, 3f).

We observed a structure similar to reticular but with much broader ridges and dimple-like depressions between them (Fig. 5a). To our knowledge, this structure has not been described from seed surfaces, and we suggest the term 'foveolate' for this type of ornamentation.

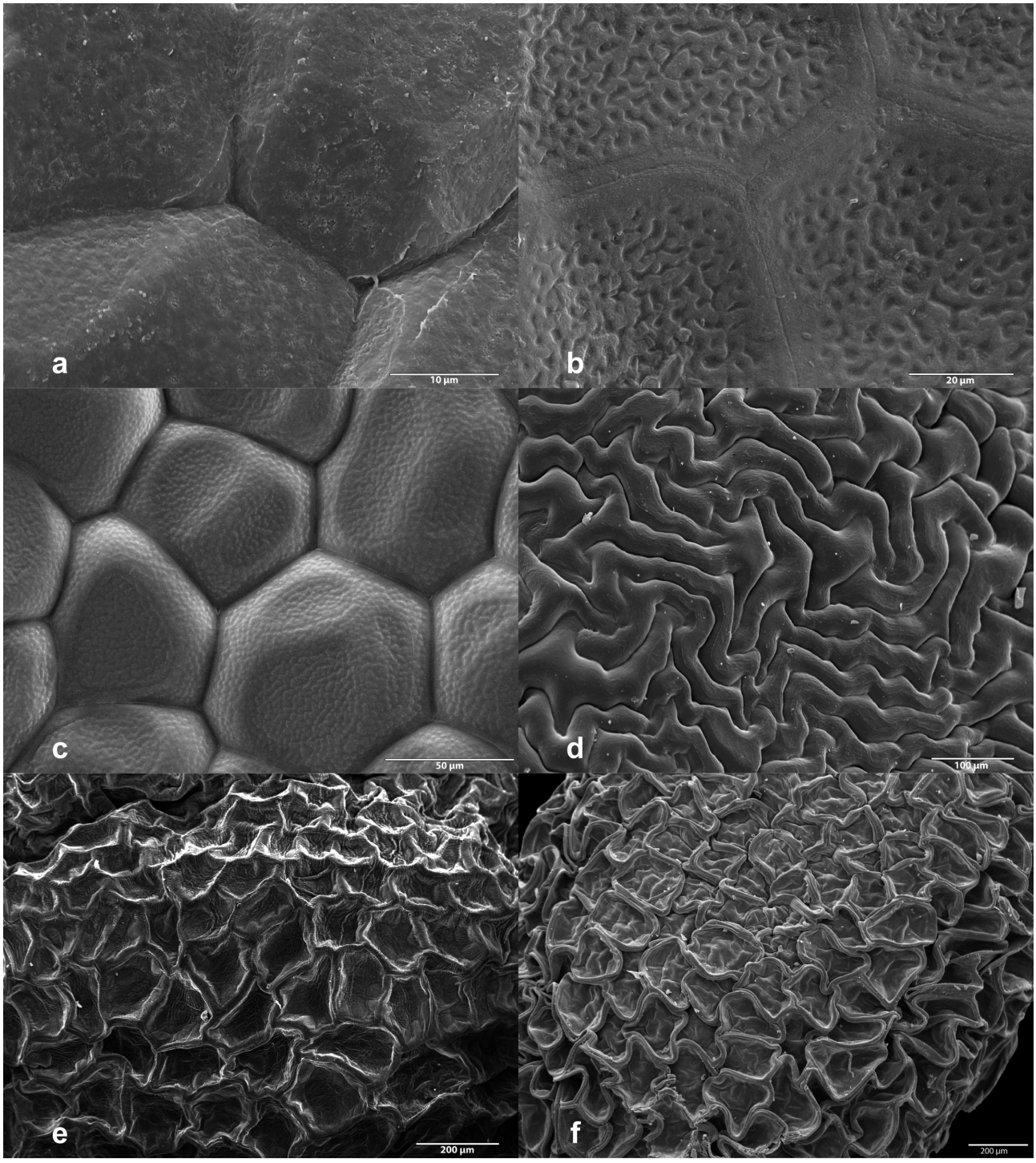

Fig. 2. (a–f) Seed surface coat details of *Hyacinthaceae*: (a) *Dipcadi ciliare* Baker, (b) *Dipcadi glaucum* Baker, (c) *Dipcadi serotinum* (L.) Medik., (d) *Avonsera convallarioides* (H. Perrier) Speta, (e) *Autonoë* sp., (f) *Ornithogalum baeticum* Boiss.

3.3. Tertiary sculpture

We found a considerable variety of waxes or rather wax-like structures on the outer seed cell surfaces. At present, the chemistry of these structures is unknown. These wax layers may cover the entire seed, in some cases hiding the primary sculptures (Fig. 5b), or they are interrupted at the anticlinal borders (Fig. 2a, 5c).

Other structures of the tertiary sculpture are not continuous. The sample *Trimelopter* sp. (05178) shows quite wide, closely recumbent rods (Fig. 3f). In *Thuranthos basuticum* the rods are helical (Fig.

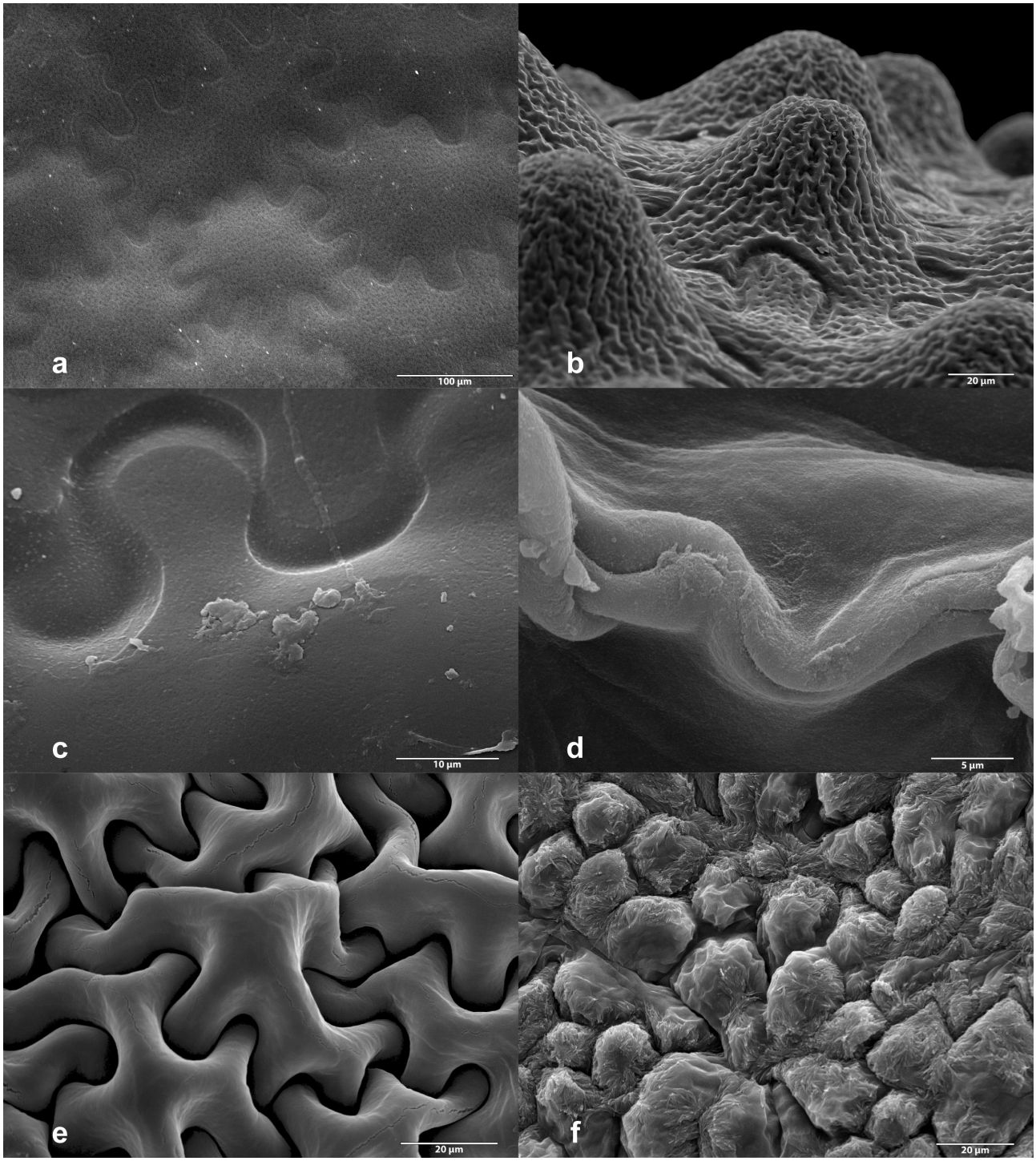

Fig. 3. (a–f) Seed surface coat details of *Hyacinthaceae*: (a) *Neopatersonia falcata* G. J. Lewis, (b) *Neopatersonia uitenhagensis* Schönland, (c) *Galtonia regalis* Hilliard & B. L. Burtt, (d) *Nicipe sardienii* (van Jaarsv.) Mart.-Azorín, M. B. Crespo & Juan, (e) *Eliokarmos* cf. *multifolius*, (f) *Trimelopter* sp.

4d). In other cases, they were more finely structured, so that the entire surface appears almost fuzzy (Fig. 5d).

Comparisons with the results of Davis 1971, Jessop 1975, Barthlott & Ehler 1977, or Huth & al. 2018 allow a cautious conclusion that it could be epicuticular waxes, but further research is necessary.

3.4. Stomata

We could locate stomata in seeds of *Boosia macrocentra*, *Avonsera convallarioides* (Fig. 5c) and

Urginavia capensis. Further research, especially about their function, is needed.

3.5. Characterization of seed morphology of genera based on the studied samples

Subfamily Ornithogaloideae

3.5.1. ***Albuca*** L.

Seed morphology: D-shaped, drop-shaped, elliptic or circular in lateral view, flattened in cross section, grey brown or black brown.

Primary sculpture: Cell area 2702–5546.7–8681.3 µm². Cell perimeter 60–121.62–196.46 µm. Frame width 75–102.05–122 µm. Frame height 336.44–453.36–570.28 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall irregularly lobate in surface view, boundaries channelled, cell corners not divergent or recessed, periclinal cell wall plane, convex, hemispherical, dome-shaped, or with several papillae.

Secondary sculpture: Surface of the outer cell wall smooth, smooth-foveolate, folded, or verrucous.

Stomata absent.

3.5.2. ***Avonsera*** Speta

Seed morphology: Drop-shaped in lateral view, circular in cross section, beige to orange.

Primary sculpture: Cell area 14109 µm². Cell perimeter 1088.8 µm. Frame width 293.75 µm. Frame height 156.25 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries channelled, cell corners not divergent, periclinal cell wall convex.

Secondary sculpture: Surface of the outer cell wall verrucous, with waxes.

Tertiary sculpture: Wax layer plane, interrupted.

Stomata present.

3.5.3. ***Battandiera*** Maire

Seed morphology: Ovate or circular in lateral view, flattened in cross section, brown or black.

Primary sculpture: Cell area 1565.3–3559.1–7206.4 µm². Cell perimeter 151.89–339.53–693.99 µm. Frame width 51–72.12–113.66 µm. Frame height 46–82.78–142.99 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall straight or irregularly lobate in surface view, boundaries channelled or plane, cell corners not divergent, periclinal cell wall plane, convex, or concave.

Secondary sculpture: Surface of the outer cell wall smooth or verrucous, sometimes with waxes.

Tertiary sculpture: Wax layer plane-smooth.

Stomata absent.

3.5.4. ***Cathissa*** Salisb.

Seed morphology: D-shaped in lateral view, irregularly edged in cross section, black.

Primary sculpture: Cell area 3495.9–3968.2–4286.5 µm². Cell perimeter 276.9–319.27–359.37 µm. Frame width 80–99.85–131.56 µm. Frame height 54–78–92 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall straight or irregularly lobate in surface view, boundaries raised or channelled, cell corners not divergent, periclinal cell wall with several collapsed papillae, hemispherical or concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall verrucous.

Stomata absent.

3.5.5. ***Coilonox*** Raf.

Seed morphology: D-shaped, drop shaped or circular in lateral view, flattened in cross section, black or grey brown.

Primary sculpture: Cell area 3233.1–5690.2–7870.8 µm². Cell perimeter 440.63–62779–498108 µm. Frame width 70.74–118.71–154.16 µm. Frame height 81.06–109.81–151.74 µm. Cell shape consistent, elongate-tetragonal or elongate-polygonal. Anticlinal cell wall irregularly lobate in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall plane, dome-shaped, or with several papillae.

Secondary sculpture: Surface of the outer cell wall verrucous.

Stomata absent.

3.5.6. ***Dipcadi*** Medik.

Seed morphology: Elliptic to circular in lateral view, flattened in cross section, black.

Primary sculpture: Cell area 910.07–8237.9 µm². Cell perimeter 120.65–351.61 µm. Frame width 39.8–103.71 µm. Frame height 36.44–121.21 µm. Cell shape consistent or inconsistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries raised or channelled, cell corners not divergent. Periclinal cell wall plane or concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall foveolate, verrucous, or cohesion deformed, sometimes with waxes.

Tertiary sculpture: Wax layer plane-verrucous.

Stomata absent.

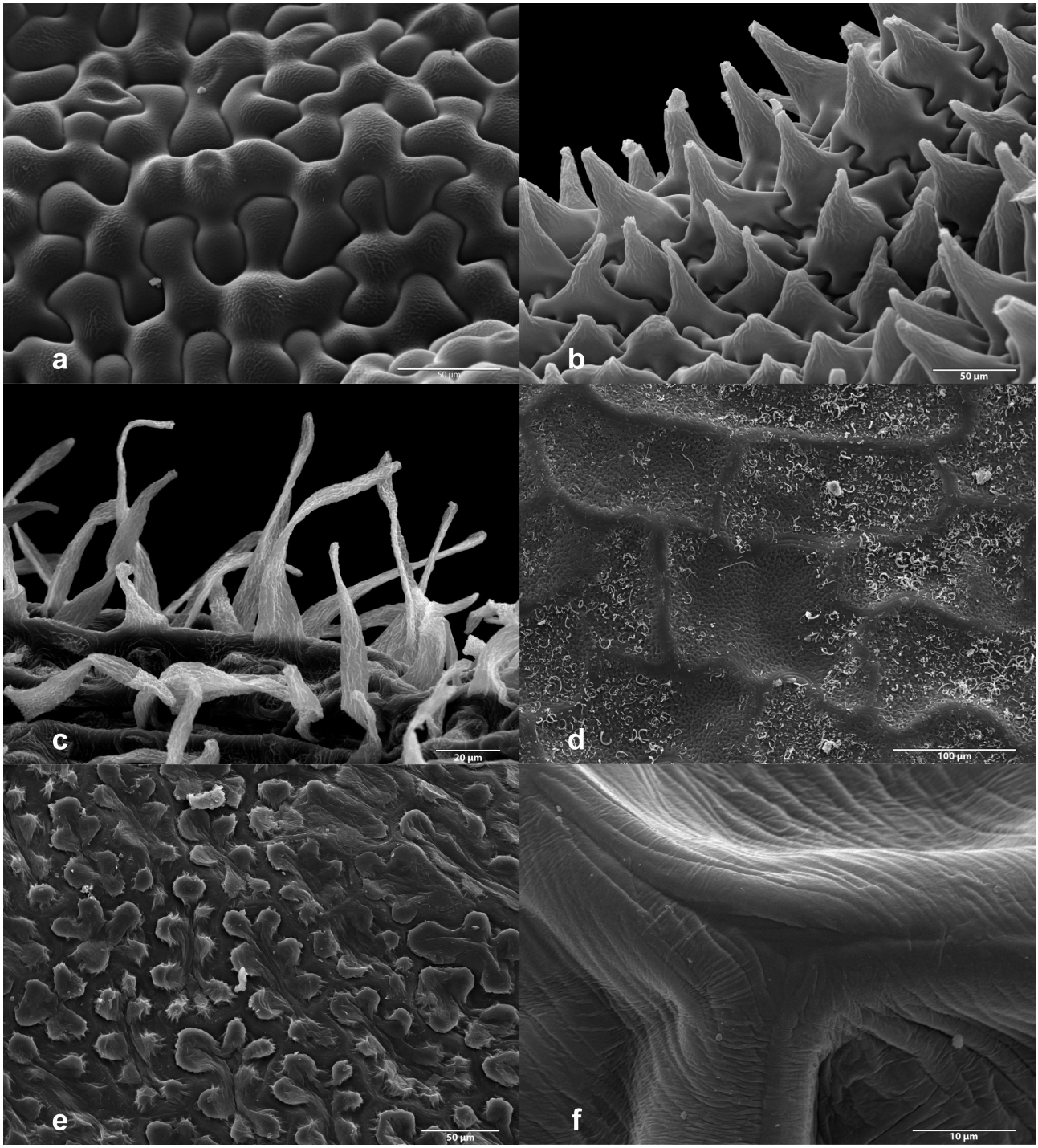

Fig. 4. (a–f) Seed surface coat details of *Hyacinthaceae*: (a) *Coilonox concordianum* (Baker) Speta, (b) *Eliokarmos decus-montium* (G. Will.) Mart.-Azorín, M. B. Crespo & Juan, (c) *Eliokarmos pilosus* (Jacq.) Mart.-Azorín, M. B. Crespo & Juan, (d) *Thuranthos basuticum* (E. Phillips) Oberm., (e) *Cathissa reverchonii* (Lange) Speta, (f) *Ornithogalum kochii* Parl.

3.5.7. ***Eliokarmos*** Raf.

Seed morphology: Drop- or D-shaped in lateral view, irregularly edged or irregularly circular in cross section, black or brown.

Primary sculpture: Cell area 121.8–3088.7–7946.7 µm². Cell perimeter 223.04–18262–214334 µm. Frame width 55.1–4173.9–49142 µm. Frame height 40.55–73.17–123.09 µm. Cell shape consistent or inconsistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall irregularly lobate in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall with one or several papillae, hemispherical or domed.

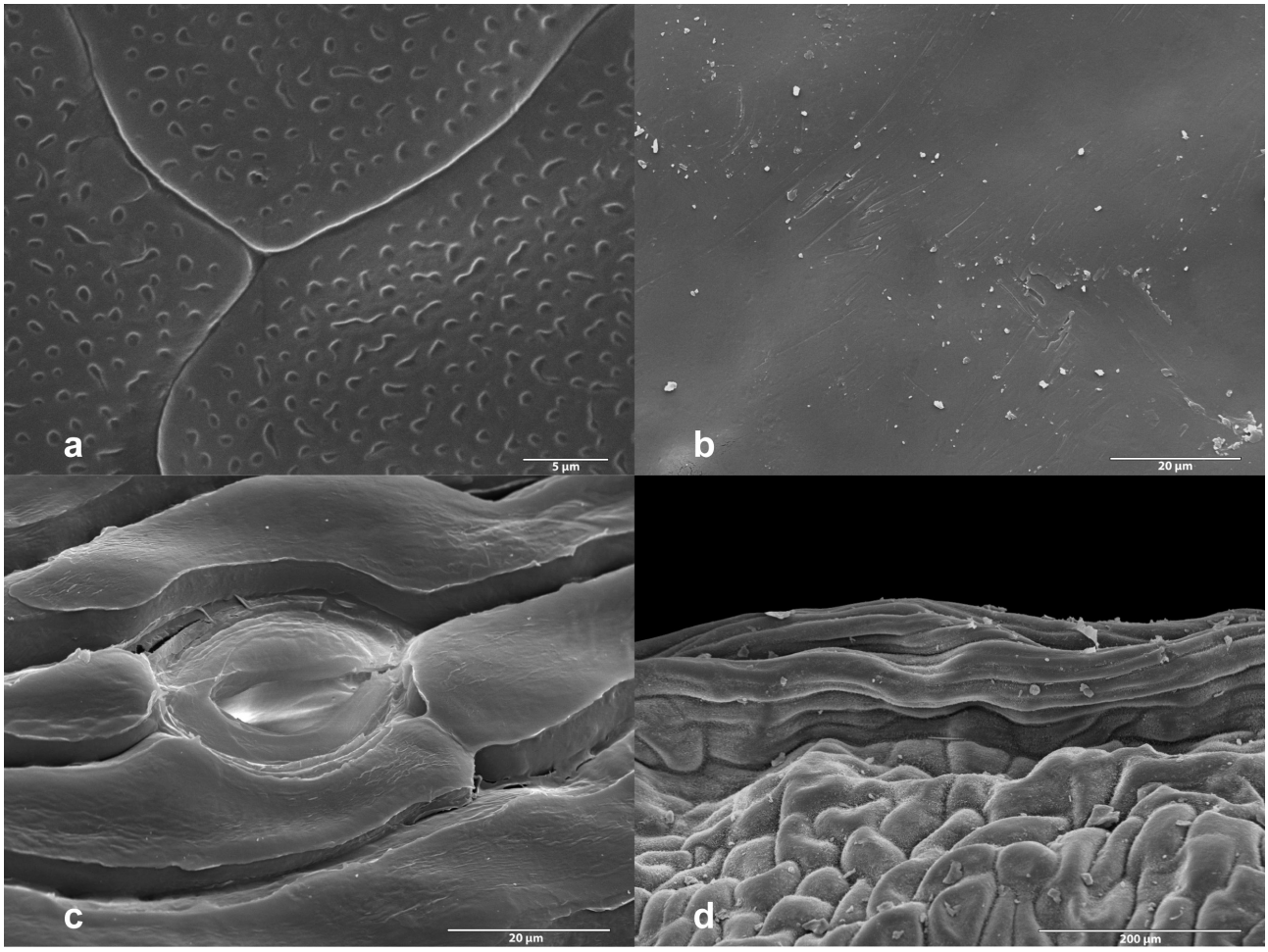

Fig. 5. (a–f) Seed surface coat details of *Hyacinthaceae*: (a) *Albuca paradoxa* Dinter, (b) *Ethesia tanquana* Mart.-Azorín & M. B. Crespo, (c) *Avonsera convallarioides* (H. Perrier) Speta, (d) *Oziroë arida* (Poepp.) Speta.

Secondary sculpture: Surface of the outer cell wall smooth, verrucous, or coarsely to medium-finely folded.

Stomata absent.

3.5.8. ***Ethesia*** Raf.

Seed morphology: Ovate or circular in lateral view, flattened in cross section, black.

Primary sculpture: Cell area 3680.5–8537.2–10966 µm². Cell perimeter 394.34–449.95–477.76 µm. Frame width 82.58–123.84–144.47 µm. Frame height 83.87–130.9–154.42 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall U-undulate in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall convex.

Secondary sculpture: Surface of the outer cell wall smooth or verrucous, sometimes with waxes.

Tertiary sculpture: Wax layer plane-smooth or plane-verrucous.

Stomata absent.

3.5.9. ***Galtonia*** Dence

Seed morphology: Elliptic to D-shaped in lateral view, tricuspidate or flattened-tricuspidate to lineal in cross section, brown.

Primary sculpture: Cell area 2517.9–7557.9–10214 µm². Cell perimeter 220.42–597.87–795.36 µm. Frame width 50.78–160.13–266.96 µm. Frame height 59.81–108.76–218.56 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall Ω-undulate or irregularly lobate in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall generally convex.

Secondary sculpture: Surface of the outer cell wall smooth, generally with waxes.

Tertiary sculpture: Wax layer plane-verrucous or plane-granulous.

Stomata absent.

3.5.10. ***Honorius*** Gray

Seed morphology: Circular in lateral view and in cross section, brown.

Primary sculpture: Cell area 3677 µm². Cell perimeter 265.29 µm. Frame width 78.95 µm. Frame height 67.37 µm. Cell shape consistent, isodiametric-tetragonal or elongate-tetragonal. Anticlinal cell wall straight or curved in surface view, boundaries raised, cell corners not divergent. Periclinal cell wall concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall smooth or medium-finely folded, with waxes.

Tertiary sculpture: Wax layer with recumbent rods or platelets.

Stomata absent.

3.5.11. ***Igidia*** Speta

Seed morphology: Drop-shaped in lateral view, irregularly edged or flattened in cross section, black or brown.

Primary sculpture: Cell area 11604–42037–96422 µm². Cell perimeter 619.67–2201.7–4815.5 µm. Frame width 194.34–348.99–607.81 µm. Frame height 172.72–271.39–426.56 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall irregularly lobate in surface view, boundaries raised or channelled, cell corners not divergent. Periclinal cell wall convex or concave.

Secondary sculpture: Surface of the outer cell wall verrucous or reticulate.

Stomata absent.

3.5.12. ***Loncomelos*** Raf.

Seed morphology: Drop- or D-shaped in lateral view, irregularly edged or irregularly circular in cross section, black or brown.

Primary sculpture: Cell area 3191.7–3298.4–3391.6 µm². Cell perimeter 240.92–263.18–299.39 µm. Frame width 60–73.91–88.85 µm. Frame height 69.09–79.33–98.75 µm. Cell shape consistent, elongate-tetragonal or elongate-polygonal. Anticlinal cell wall straight, undulate, or irregularly lobate in surface view, boundaries raised or channelled, cell corners not divergent. Periclinal cell wall convex, concave, or concave-cohesion deformed with several papillae.

Secondary sculpture: Surface of the outer cell wall medium-finely folded, verrucous, or cohesion deformed.

Stomata absent.

3.5.13. ***Melomphis*** Raf.

Seed morphology: Irregularly elliptic in lateral view, flattened in cross section, black.

Primary sculpture: Cell area 1210.5 µm². Cell perimeter 545.8 µm. Frame width 195.88 µm. Frame height 129.75 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall with several papillae, convex or plane.

Secondary sculpture: Surface of the outer cell wall coarsely folded or cohesion deformed.

Stomata absent.

3.5.14. ***Neopatersonia*** Schönland

Seed morphology: Drop- or D-shaped in lateral view, flattened or circular in cross section, black.

Primary sculpture: Cell area 4726.7–11596–16964 µm². Cell perimeter 452.81–657.97–815.03 µm. Frame width 112.06–159.23–192.4 µm. Frame height 88.44–132.02–163.15 µm. Cell shape consistent, basic shape of the cells generally elongate-polygonal. Anticlinal cell wall U-undulate in surface view, Ω-undulate or irregularly lobate, boundaries channelled, cell corners not divergent. Periclinal cell wall convex or plane with several papillae.

Secondary sculpture: Surface of the outer cell wall smooth, generally medium-finely folded, or verrucous.

Stomata absent.

3.5.15. ***Nicipe*** Raf.

Seed morphology: Drop- or D-shaped to elliptic in lateral view, flattened or irregularly edged to irregularly circular in cross section, black or brown.

Primary sculpture: Cell area 711.29–2309.7–4434.9 µm². Cell perimeter 129.57–276.58–559.93 µm. Frame width 24.95–71.98–155.26 µm. Frame height 44.46–64.59–112.48 µm. Cell shape consistent, elongate-tetragonal or elongate-polygonal. Anticlinal cell wall straight or irregularly lobate in surface view, boundaries raised or channelled, cell corners not divergent. Periclinal cell wall plane, concave-cohesion deformed, or domed with one or several papillae up to trichomes.

Secondary sculpture: Surface of the outer cell wall smooth, coarsely folded, verrucous, or cohesion deformed.

Stomata absent.

3.5.16. ***Ornithogalum*** L.

Seed morphology: Drop-shaped to elliptic in lateral view, elliptic to circular in cross section, brown.

Primary sculpture: Cell area 10379–16927–26353 µm². Cell perimeter 446.68–557.45–683.19 µm. Frame width 108.42–167.9–223.44 µm. Frame height 98.44–161.5–196.66 µm. Cell shape consistent, isodiametric-tetragonal, elongate-tetragonal, isodia-

metric-polygonal, or elongate-polygonal. Anticlinal cell wall straight or curved in surface view, boundaries raised, cell corners not divergent. Periclinal cell wall concave or concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall smooth or finely folded, generally cohesion deformed, striate or with waxes.

Tertiary sculpture: Wax layer with blisters.

Stomata absent.

3.5.17. ***Pseudogaltonia*** Kuntze

Seed morphology: Elliptic to circular in lateral view, flattened in cross section, black.

Primary sculpture: Cell area 2843.1–3879.6–4916.1 µm². Cell perimeter 230.17–258.88–287.58 µm. Frame width 86.32–100.32–114.31 µm. Frame height 60.13–64.51–68.89 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries channelled or plane, cell corners not divergent. Periclinal cell wall concave or plane.

Secondary sculpture: Surface of the outer cell wall smooth or verrucous, generally with waxes.

Tertiary sculpture: Wax layer plane-smooth or plane-verrucous.

Stomata absent.

3.5.18. ***Stellarioides*** Medik.

Seed morphology: D-shaped in lateral view, tricuspidate in cross section, black-brown.

Primary sculpture: Cell area 2843.1–3879.6–4916.1 µm². Cell perimeter 230.17–258.88–287.58 µm. Frame width 86.32–100.32–114.31 µm. Frame height 60.13–64.51–68.89 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall irregularly lobate in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall generally hemispherical to domed.

Secondary sculpture: Surface of the outer cell wall smooth, verrucous, or reticulate, rarely with waxes.

Tertiary sculpture: Wax layer plane-verrucous.

Stomata absent.

3.5.19. ***Trimelopter*** Raf.

Seed morphology: D-shaped or circular in lateral view, tricuspidate or flattened in cross section, black-brown.

Primary sculpture: Cell area 2144.4–3129.5–4621.1 µm². Cell perimeter 260.88–330.89–442.06 µm. Frame width 62.3–81.05–105.26 µm. Frame height 63.91–70.57–80.53 µm. Cell shape consistent. Basic shape of the cells generally elongate-polygonal. Anticlinal cell wall irregularly lobate in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall convex or with several papillae.

Secondary sculpture: Surface of the outer cell wall cohesion deformed, sometimes with waxes.

Tertiary sculpture: Wax layer with recumbent rods.

Stomata absent.

Subfamily *Hyacinthoideae*

3.5.20. ***Autonoë*** (Webb & Berthel.) Speta

Seed morphology: D-shaped in lateral view, elliptic or circular to irregularly circular in cross section, black-brown.

Primary sculpture: Cell area 19319 µm². Cell perimeter 557 µm. Frame width 176 µm. Frame height 110 µm. Cell shape consistent, generally isodiametric-polygonal. Anticlinal cell wall straight in surface view, cell corners not divergent, periclinal cell wall concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall finely folded.

Stomata absent.

3.5.21. ***Barnardia*** Lindl.

Seed morphology: Ovate to elliptic or oblong in lateral view, irregularly edged in cross section, brown.

Primary sculpture: Cell area 1383.7–7487 µm². Frame width 57.88–269 µm. Frame height 36.7–46 µm. Cell perimeter 557.12–576 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal boundaries raised or channelled, cell corners not divergent, periclinal cell wall concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall verrucous, sometimes with waxes.

Tertiary sculpture: Wax layer plane-verrucous.

Stomata absent.

3.5.22. ***Chouardia*** Speta

Seed morphology: Elliptic or drop-shaped in lateral view, irregularly D-shaped in cross section, brown.

Primary sculpture: Cell area 1343 µm². Cell perimeter 149 µm. Frame width 48 µm. Frame height 39 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries raised, cell corners not divergent. Periclinal cell wall concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall cohesion deformed and folded.

Stomata absent.

3.5.23. ***Schnarfia*** Speta

Seed morphology: Ovate in lateral view, circular in cross section, beige to orange.

Primary sculpture: Cell area 631.24 µm². Cell perimeter 106.74 µm. Frame width 37.94 µm. Frame height 26.79 µm. Cell shape consistent, isodiametric-tetragonal, elongate-tetragonal, isodiametric-polygonal, or elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries raised, cell corners not divergent. Periclinal cell wall concave or concave-cohesion deformed.

Stomata absent.

Subfamily *Urgineoideae*

3.5.24. ***Boosia*** Speta

Seed morphology: Irregularly oblong in lateral view, irregularly edged in cross section, grey brown.

Primary sculpture: Cell area 13210 µm². Cell perimeter 587.1 µm. Frame width 245.9 µm. Frame height 113.8 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries channelled, cell corners not divergent, periclinal cell wall concave-cohesion deformed

Secondary sculpture: Surface of the outer cell wall smooth or cohesion deformed.

Stomata present.

3.5.25. ***Bowiea*** Harv. ex Hook.f.

Seed morphology: Irregularly oblong in lateral view, irregularly circular in cross section, grey brown.

Primary sculpture: Cell area 5986.1 µm². Cell perimeter 343.95 µm. Frame width 129.17 µm. Frame height 75.26 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall irregularly lobate in surface view, boundaries raised, cell corners recessed, periclinal cell wall convex.

Secondary sculpture: Surface of the outer cell wall smooth.

Stomata absent.

3.5.26. ***Charybdis*** Speta

Seed morphology: Elliptical in lateral view, irregularly circular in cross section, grey brown.

Primary sculpture: Cell area 19753 µm². Cell perimeter 731.46 µm. Frame width 278 µm. Frame height 127.83 µm. Cell shape inconsistent.

Secondary sculpture: Surface of the outer cell wall smooth.

Stomata absent.

3.5.27. ***Drimia*** Jacq. ex Willd.

Seed morphology: Elliptical to reniform in lateral view, flattened and winged in cross section, brown.

Primary sculpture: Cell area 5980.6–7864.7 µm². Cell perimeter 348.74–404 µm. Frame width 137.67–168.45 µm. Frame height 65.56–67.46 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall smooth or cohesion deformed.

Stomata absent.

3.5.28. ***Fusifilum*** Raf.

Seed morphology: Elliptical to D-shaped in lateral view, irregularly circular and winged in cross section, brown.

Primary sculpture: Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall concave.

Secondary sculpture: Surface of the outer cell wall smooth.

Stomata absent.

3.5.29. ***Litanthus*** Harv.

Seed morphology: Triangular in lateral view, irregularly edged in cross section, brown.

Primary sculpture: Cell area 1020.8 µm². Cell perimeter 136.72 µm Frame width 45.92 µm. Frame height 33.14 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall hemispherical

Secondary sculpture: Surface of the outer cell wall coarsely folded and cohesion deformed.

Stomata absent.

3.5.30 ***Mucinaea*** M. Pinter, Mart.-Azorín, U. Müll.-Doblies, D. Müll.-Doblies, Pfosser & Wetschnig

Seed morphology: Flattened D-shaped in lateral view, irregularly circular and winged in cross section, brown.

Primary sculpture: Cell area 12167 µm². Cell perimeter 420.2 µm. Frame width 142.45 µm.

Frame height 113.8 µm. Cell shape consistent. Basic shape of the cells generally isodiametric-polygonal. Anticlinal cell wall straight in surface view, boundaries raised, cell corners not divergent. Periclinal cell wall concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall smooth.

Stomata absent.

3.5.31. ***Rhadamanthopsis*** (Oberm.) Speta

Seed morphology: Irregularly rectangular or flattened D-shaped in lateral view, irregularly circular to ovate and winged in cross section, grey-brown.

Primary sculpture: Cell area 8174.4 µm². Cell perimeter 409.13 µm. Frame width 143.1 µm. Frame height 90.52 µm. Cell shape consistent, generally isodiametric-polygonal. Anticlinal cell wall curved in surface view, boundaries channelled or plane, cell corners not divergent. Periclinal cell wall convex or plane.

Secondary sculpture: Surface of the outer cell wall reticulate.

Stomata absent.

3.5.32. ***Rhadamanthus*** Salisb.

Seed morphology: Flattened D-shaped or elliptical in lateral view, irregularly circular to ovate and winged in cross section, brown.

Primary sculpture: Cell area 3400.3–4200 µm². Cell perimeter 279.43–313.02 µm. Frame width 91.74–129.95 µm. Frame height 54.28–76.2 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall straight in surface view, undulate, curved, or irregularly lobate, boundaries raised or plane, cell corners not divergent. Periclinal cell wall convex or concave.

Secondary sculpture: Surface of the outer cell wall smooth or foveolate.

Stomata absent.

3.5.33. ***Rhodocodon*** Baker

Seed morphology: D-shaped or elliptical to drop-shaped or lanceolate in lateral view, elliptical to flattened, irregularly circular or irregularly edged, sometimes winged in cross section, black or brown.

Primary sculpture: Cell area 5356.3–24409 µm². Cell perimeter 364.72–1187.3 µm. Frame width 128.2–370.8 µm. Frame height 80.59–280.62 µm. Cell shape consistent, elongate-tetragonal or elongate-polygonal. Anticlinal cell wall straight or irregularly lobate in surface view, boundaries raised, channelled or plane, cell corners not divergent or recessed. Periclinal cell wall plane, convex, concave, or concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall smooth, foveolate, verrucous, or reticulate, mostly with waxes.

Tertiary sculpture: Wax layer plane-smooth or plane-verrucous.

Stomata absent.

3.5.34. ***Schizobasis*** Baker

Seed morphology: Flattened or lanceolate in lateral view, elliptical to flattened in cross section, brown.

Primary sculpture: Cell area 9856.1 µm². Cell perimeter 612.27 µm. Frame width 254.54 µm. Frame height 103.6 µm. Cell shape consistent, elongate-tetragonal or elongate-polygonal. Anticlinal cell wall straight in surface view, boundaries raised or channelled, cell corners not divergent. Periclinal cell wall convex or plane.

Secondary sculpture: Surface of the outer cell wall foveolate.

Stomata absent.

3.5.35. ***Sekanama*** Speta

Seed morphology: Irregularly elliptical to irregularly circular in lateral view, elliptical to flattened and winged in cross section, brown.

Primary sculpture: Cell area 8944.6 µm². Cell perimeter 500.6 µm. Frame width 163.42 µm. Frame height 105 µm. Cell shape consistent, generally isodiametric-polygonal. Anticlinal cell wall undulate in surface view, boundaries raised, cell corners not divergent. Periclinal cell wall concave.

Secondary sculpture: Surface of the outer cell wall verrucous, with waxes.

Stomata absent.

3.5.36. ***Tenicroa*** Raf.

Seed morphology: Irregularly elliptical to irregularly circular in lateral view, circular or elliptical and winged in cross section, brown.

Primary sculpture: Cell area 11236 µm². Cell perimeter 962.66 µm. Frame width 191.56 µm. Frame height 154.38 µm. Cell shape consistent, generally elongate-polygonal. Anticlinal cell wall irregularly lobate in surface view, boundaries channelled, cell corners not divergent. Periclinal cell wall convex.

Secondary sculpture: Surface of the outer cell wall verrucous.

Stomata absent.

3.5.37. ***Thuranthos*** C.H.Wright

Seed morphology: Irregularly elliptical to irregularly circular in lateral view, flattened-elliptical and winged in cross section, black.

Primary sculpture: Cell area 9152.8 µm². Cell perimeter 493.54 µm. Frame width 493.54 µm. Frame height 163.09 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall curved in surface view, boundaries plane, cell corners not divergent. Periclinal cell wall concave.

Secondary sculpture: Surface of the outer cell wall verrucous, with waxes.

Tertiary sculpture: Wax layer with spirally coiled rods.

Stomata absent.

3.5.38. ***Urginavia*** Speta

Seed morphology: Irregularly elliptical to D-shaped in lateral view, circular or elliptical and winged in cross section, brown.

Primary sculpture: Cell area 9540.8–19282 µm². Cell perimeter 440.1–800.45 µm. Frame width 139.98–214.21 µm. Frame height 99.79–173.16 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall undulate or curved in surface view, boundaries raised, channelled or plane, cell corners not divergent. Periclinal cell wall concave or concave-cohesion deformed.

Secondary sculpture: Surface of the outer cell wall smooth, verrucous, or with waxes.

Tertiary sculpture: Wax layer with platelets.

Stomata absent.

3.5.39. ***Urginea*** Steinh.

Seed morphology: Irregularly elliptical to D-shaped or flattened to lanceolate in lateral view, circular to elliptical and winged, or irregularly edged in cross section, brown.

Primary sculpture: Cell area 4108.3–10198 µm². Cell perimeter 344.89–492.02 µm. Frame width 129.3–176.42 µm. Frame height 74.01–96.07 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall curved in surface view, boundaries raised or channelled, cell corners not divergent. Periclinal cell wall concave or convex.

Secondary sculpture: Surface of the outer cell wall smooth to folded or verrucous, sometimes with waxes.

Tertiary sculpture: Wax layer with recumbent rods.

Stomata absent.

Subfamily *Oziroëoideae*

3.5.40. ***Oziroë*** Raf.

Seed morphology: Irregularly elliptical to D-shaped or flattened to lanceolate in lateral view, irregularly circular in cross section, grey-brown.

Primary sculpture: Cell area 2918.1–6301.1 µm². Cell perimeter 243.04–425.66 µm. Frame width 64.19–94.6 µm. Frame height 79.51–147.36 µm. Cell shape consistent, isodiametric-polygonal or elongate-polygonal. Anticlinal cell wall straight in surface view, curved or irregularly lobate, boundaries channelled, cell corners not divergent. Periclinal cell wall hemispherical.

Secondary sculpture: Surface of the outer cell wall with waxes.

Tertiary sculpture: Wax layer with platelets.

Stomata absent.

4. Discussion

4.1. Classification on subfamily level

Clear systematic inferences cannot be established at subfamily level. There are some tendencies, such as the lack of convex periclinal outer cell walls and the absence of undulate or irregularly lobate anticlinal walls in the studied samples of *Hyacinthoideae*, or that the more elongate convex cell walls (domed, papillate, hair-papillate, and trichomes) are more common in *Ornithogaloideae* (Table 2). Apart from these few distinctions, however, no valid syndromes can be derived based on these features.

4.2. Classification on tribe level

A differentiation at the level of tribes is much more practicable (Table 4, 5). Martínez-Azorín & al. 2011 found three main clades corresponding to the previously recognized tribes *Albuceae*, *Dipcadieae* and *Ornithogaleae*. Martínez-Azorín & al. 2011: 9 noted that puzzle-like testae are widespread in the subfamily *Ornithogaloideae*. Their assumption that most species of the tribes *Albuceae*, *Dipcadieae* and *Ornithogaleae* excepting a few taxa will show puzzle-like structures is now confirmed.

In part such cell clusters can be recognized well with a clear eye. A puzzle-like structure is due to the lobate and undulate, elongate-polygonal cells but also larger isodiametric cells with a distinctly convex or concave periclinal outer cell wall appear similar. The SEM investigation shows that *Dipcadieae* possess exclusively straight anticlinal (Fig. 2a, b, c) cell walls. *Albuceae*, in contrast to *Ornithogaleae*, lack U- and Ω-undulate or curved walls (Fig. 5a). All investigated *Albuceae* possess ir-

regularly lobate anticlinal walls except *Battandiera amoena*. In *Ornithogaleae* irregularly lobate types dominate. However, straight cell walls are also very common in this tribe.

Flat anticlinal boundaries and generally plane periclinal outer cell walls do not occur in *Ornithogaleae*, but hair-papillate structures (Fig. 4b) or trichomes (Fig. 4c) are found exclusively in *Ornithogaleae*. In addition, it should be noted that the genera *Ornithogalum* and *Honorius* have concave or concave-cohesion deformed cells, and *Ethesia* and *Galtonia* simple convex cells (Fig. 3c). Therefore the investigated species of these genera stand out clearly from the remaining *Ornithogaleae* with pronouncedly convex forms. In *Albuceae* we could find all convex structures, except hair-papillae or trichomes; flat periclinal cell walls could be located as well. Except for *Dipcadi viride*, convex periclinal outer cell walls are completely absent in *Dipcadieae* where concave or cohesion deformed cells dominate.

Regarding the secondary structure, it is striking that reticulate (Fig. 2b) and foveolate (Fig. 5a) structures only occur in *Albuceae* and *Dipcadieae*.

4.3. Classification on genus level

Unambiguous syndromes for particular genera cannot be clearly identified. Common areas are found mainly in the primary structure. The majority of *Ornithogaloideae* show uniformly elongate-polygonal cell patterns. Comparing the phylogenetic results of Martínez-Azorín & al. 2011, there are clear differences:

While *Cathissa*, *Melomphis* and *Neopatersoni*a species possess only elongate-polygonal cells, *Honorius*, *Ornithogalum*, *Nicipe* and *Loncomelo*s species show mixed forms. Likewise, the genus *Galtonia* can be distinguished from *Eliokarmos* by clearly isodiametric cells (except *Eliokarmos decusmontium*, which has isodiametric and elongate cell types) and from *Ethesia* with generally elongate-polygonal cells. *Coilonox* additionally forms tetragonal cells, in contrast to the generally elongate-polygonal cell types of the genera *Igidia*, *Stellarioides* and *Albuca*. The genera *Battandiera* and *Dipcadi* are characterized by species with isodiametric- or elongate-polygonal cells (Fig. 2b, c) which differentiate them from *Trimelopter* and *Pseudogaltonia*, both with exclusively elongate-polygonal cells. Uniformly elongate-polygonal cells are found in all studied species of *Tenicroa*, *Bowiea* and *Boosia*.

As mentioned above (4.1.), testae with undulate anticlinal walls are only found in *Ornithogaleae*. All examined *Ethesia* and *Neopatersonia* (Fig. 3a) species possess U-undulate anticlinal walls, but Ω-undulate forms also occur in *Neopatersonia* (Fig. 3b), though less frequently. Uniformly Ω-undulate anticlinal walls are only found in *Galtonia regalis* (Fig. 3c).

Secondary sculptures suggest systematic implications in some genera. Exclusively verrucous surfaces can be found in *Barnardia*, *Cathissa*, *Coilonox* and *Sekanama*. The seeds of *Bowiea*, *Charybdis* and *Neopatersonia* are always smooth. However, other genera comprise species with verrucous and species with smooth seeds, together with species showing other secondary structures. The situation with the tertiary structure, i.e. the presence of wax-like substances is similar. Only the investigated species in *Galtonia* and *Pseudogaltonia* all exhibit waxes, albeit in different forms. In our opinion, secondary and tertiary sculptures are definitely helpful and diagnostic at species level.

4.4. Morphological characters

The enormous variability of the seed surface of *Hyacinthaceae* is astonishing. For example, we expected a more significant number of concave (Fig. 1g, 2b, c, e, f, 4c) structures. Surprisingly, convex (Fig. 1f, 2d, 3b, e, f, 4a–c) cell walls occur more often, despite the dry testa (Table 2). In contrast, the undulate (Fig. 3a–c) or irregularly lobate (Fig. 3e, 4a) anticlinal cell walls, which are said to have increased tear resistance, were to be expected (Table 2).

Stomata on testae seem to be a rare phenomenon. Within the *Hyacinthaceae*, Wetschnig & al. 2002 could find stomata on *Ledebouria floribunda* (Baker) Jessop. Stomata could not be found in any of the examined *Ornithogalum* species, contrary to the observation of Netolitzky 1926 who wrote that stomata do occur in *Ornithogalum*.

Waxes are not uncommon on testae in *Hyacinthaceae*. We agree with Barthlott & Ehler 1977 that waxes only have a subordinate role for systematics. We could not identify any significant syndromes on subfamily and tribe level. Nevertheless, they play an extraordinary role at species level (Brudermann & al. 2018).

In general, the structures found during our SEM investigations are consistent with those described by Barthlott & Ehler 1977 and Barthlott 1981, 1984. Furthermore, we can add the term foveolate (Fig. 5a) to the current terminology.

Our investigations reveal a great variety of morphological characteristics of the seed surface of *Hyacinthaceae*. The present work shows that a synoptical treatment of the various characters can make a significant contribution to classification at various taxonomic levels.

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References

APG 2003. An update of the Angiosperm Phylogeny Group Classification for the orders and families of flowering plants: APG II. – Botanical Journal of the Linnean Society 141: 99–436. <http://dx.doi.org/10.1046/j.1095-8339.2003.t01-1-00158.x>.

APG 2009. An update of the Angiosperm Phylogeny Group Classification for the orders and families of flowering plants: APG III. – Botanical Journal of the Linnean Society 161: 105–121. <https://doi.org/10.1111/j.1095-8339.2009.00996.x>.

APG 2016. An update of the Angiosperm Phylogeny Group Classification for the orders and families of flowering plants: APG IV. – Botanical Journal of the Linnean Society 181: 1–20. <https://doi.org/10.1111/boj.12385>.

Barthlott W. 1981. Epidermal and seed surface applicability and some evolutionary aspects. – Nordic Journal of Botany 1: 345–355.

Barthlott W. 1984. Microstructural features and seed surface. – In: Heywood V. & Moore D. M. (eds.). Current concepts in plant taxonomy, p. 95–105. – Academic Press; London.

Barthlott W. & Ehler N. 1977. Raster-Elektronenmikroskopie der Epidermis-Oberflächen von Spermatophyten. – Tropische und Subtropische Pflanzenwelt 19: 1–110.

Bednorz L. & Czarna A. 2008. SEM and stereoscope microscope observations on the seeds of some *Ornithogalum* (*Hyacinthaceae*) species. – Biologia 63(5): 642–646.

Brudermann A., Martínez-Azorín M., Knirsch W. & Wetschnig W. 2018. Seed morphology of *Rhodocodon* (*Hyacinthaceae*) and its systematic implications – Phyton 58(2): 139–154.

Coşkunçelebi K., Kandemir A. & Beyazoğlu O. 2000. Scanning electron microscopic examination of the seeds of *Ornithogalum* (*Liliaceae*) species distributed in Black Sea Region of Turkey. – Biologia 55(4): 397–401.

Davis D. G. 1971. Scanning electron microscopic studies of wax formations on leaves of higher plants – Canadian Journal of Botany 49(4): 543–546.

Huth M. A., Huth A. & Koch K. 2018. Morphological diversity of β-diketone wax tubules on *Eucalyptus gunnii* leaves and real time observation of self-healing of defects in the wax layer – Australian Journal of Botany 66(4): 313–324. <http://doi.org/10.1071/BT18035>.

Jessop J. P. 1975. Studies in the bulbous *Liliaceae* in South Africa: 5. Seed surface characters and generic groupings. – Journal of South African Botany 41(2): 67–85.

Koller C. 2010. Rasterelektronenmikroskopische Untersuchungen zur Mikromorphologie von Samen der Tribus *Ornithogaleae* (*Scilloideae*, *Asparagacea*e). – Magisterarbeit, Karl-Franzens-Universität Graz.

Luidold A. K. 2010. Rasterelektronenmikroskopische Untersuchungen der Mikromorphologie von Samen der Triben *Urgineeae* und *Oziroëeae* (*Scilloideae*, *Asparagaceae*). – Magisterarbeit, Karl-Franzens-Universität Graz.

Manning J. C., Goldblatt P. & Fay M. F. 2004. A revised generic synopsis of *Hyacinthaceae* in Sub-Saharan Africa, based on molecular evidence, including new combinations and the new tribe *Pseudoprospereae*. – Edinburgh Journal of Botany 60: 533–568.

Martínez-Azorín M., Crespo M. B., Juan A. & Fay M. F. 2011. Molecular phylogenetics of subfamily *Ornithogaloideae* (*Hyacinthaceae*) based on nuclear and plastid DNA regions, including a new taxonomic arrangement. – Annals of Botany 107: 1–37.

Moret J., Couderc H., Bari A. & Delarue Y. 1990. Micromorphology of seeds of *Ornithogalum* (*Hyacinthaceae*) in North Africa. – Nordic Journal of Botany 9: 461–468.

Netolitzky F. 1926. Anatomie der Angiospermen-Samen. – In: Linsbauer K. (ed.). Handbuch der Pflanzenanatomie. (II. Abt., 2. Teil: Pteridophyten und Anthophyten). Band X. – Gebr. Borntraeger; Berlin.

Pfosser M. & Speta F. 1999. Phylogenetics of *Hyacinthaceae* based on plastid DNA sequences. – Annals of the Missouri Botanical Garden 86: 852–875.

Pfosser M., Wetschnig W., Ungar S. & Prenner G. 2003. Phylogenetic relationships among genera of *Massonieae* (*Hyacinthaceae*) inferred from plastid DNA and seed morphology. – Journal of Plant Research 116: 115–132.

Rafinesque C. S. 1837. Flora Telluriana 3. – H. Probasco; Philadelphia, PA. 100 pp.

Speta F. 1998a. *Hyacinthaceae*. – In: Kubitzki K. (ed.). The Families and Genera of Vascular Plants. III. Flowering Plants. Monocotyledons. *Lilianae* (except *Orchidaceae*), p. 261–285. – Springer; Berlin, Heidelberg.

Speta F. 1998b. Systematische Analyse der Gattung *Scilla* L. s.l. (*Hyacinthaceae*). – Phyton (Horn, Austria) 38(1): 1–141.

Werker E. 1997. Seed anatomy. – In: Fink S. & al. (eds.). Handbuch der Pflanzenanatomie / Encyclopedia of Plant Anatomy. 2. Auflage. Band X, Teil 3. – Gebr. Borntraeger; Berlin, Stuttgart.

Wetschnig W., Pfosser M. & Prenner G. 2002. Zur Samenmorphologie der *Massonieae* Baker 1871 (*Hyacinthaceae*). – Stapfia 80: 349–379.

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